Studies on Proteins, Glycoproteins and Mucopolysaccharides in Normal Human Urine

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The present paper is a summary of the following communications:

I. Berggård, I.: Proteins, glycoproteins and mucopolysaccharides in normal human urine. I. Fractionation of non-dialyzable materials by ultrafiltration and by zone electrophoresis. *Arkiv Kemi 18,* 291 (1961).

II. Berggård, I.: Proteins, glycoproteins and mucopolysaccharides in normal human urine. II. Further zone electrophoretic studies on non-ultrafilterable materials. *Arkiv Kemi 18,* 315 (1961).

III. Berggård, I.: Studies on the plasma proteins in normal human urine. *Clin. Chim. Acta 6,* 413 (1961).

IV. Berggård, I.: On a γ-globulin of low molecular weight in normal human plasma and urine. *Clin. Chim. Acta 6,* 545 (1961).

V. Berggård, I. and Risinger, C.: Quantitative immunochemical determination of albumin in normal human urine. *Acta Soc. Med. Upsaliensis 66,* 217 (1961).

References to these papers will be made using the Roman figures I–V.

Introduction

In recent years increasing interest has been devoted to studies of proteins, glycoproteins and mucopolysaccharides occurring in human urine under normal and pathological conditions. The information hitherto available on the types and amounts of these substances in normal urine is, however, very limited. Moreover, as regards the glycoproteins and mucopolysaccharides, there is only very scanty knowledge concerning the causes and significance of the increased excretion of these materials reported for a number of pathological conditions.

Investigations of the plasma proteins in normal urine can be regarded

as important from many points of view. At present it is generally assumed that the plasma proteins in urine originate from a process of glomerular filtration (1, 2) followed by non-selective tubular reabsorption (3). Provided that this theory is correct, a qualitative analysis of the plasma proteins in normal urine may give information about the permeability of the glomerular filter. Quantitative determination of these materials is the prerequisite for closer studies of functional proteinurias, e.g. that which follows physical exercise. In addition, determination of the normal plasma proteinuria might permit detection of moderately increased excretion of plasma proteins in pathological conditions where the proteinurias are not accessible to the qualitative and quantitative tests used at present in the clinic. In addition plasma proteins of low molecular weight might be more easily detected in urine than in plasma. It appears that some glycoprotein hormones, such as the gonadotropines (4) and erythropoietin (5), are more readily isolated from urine than from plasma.

An increased excretion of glycoprotein and glycopeptide materials has been demonstrated in a number of pathological states. As early as 1900 Ehrström (6) described the urinary excretion of "carbohydrate-group-rich deutero-albumos B" as a characteristic phenomenon of pathological conditions involving fever. Many decades later Baar (7) isolated a glycopeptide fraction from the urine of patients suffering from burns. Anderson and Maclagan (8) precipitated a carbohydrate-rich fraction from normal urine by means of benzoic acid. Together with Lockey these investigators observed an increased excretion of this fraction after surgical operations and in patients with cancer, collagen or inflammatory diseases (9). Increased urinary excretion of non-dialyzable hexosamine and hexose was reported for similar conditions by Hamerman *et al.* (10). Masamune *et al.* (11) have detected a urinary glycopeptide fraction which they claim is characteristic for cancer.

It is known that normal urine contains non-dialyzable and dialyzable toxic substances which give rise to effects such as leukocytosis, leukopenia or rise of temperature (cf. Westphal *et al.*, ref. 12). Such toxic substances are excreted in considerably increased quantities in certain pathological conditions, especially those associated with acute cell disintegration (e.g. burns, leukemia). Westphal *et al.* regarded the toxic substances as being of peptide character and separated them into a water-soluble and a phenol-soluble fraction. The water-soluble fraction has been reported to comprise mainly carbohydrate and, in certain pathological conditions such as leukemia, to have a much increased content of sialic acid (Kickhöfen: personal communication to Probst *et al.*, ref. 13). The amount of the toxic substances in urine also increases immediately after surgical operations (13, 14). It has been shown that when the post-operative leukocytosis and rise of temperature

are decreasing there is in the plasma a simultaneous increase of the concentrations of α_2-globulins and "polysaccharides", which usually attain a maximum about 5 days after the operation (13, 14). The results of these and other investigations might indicate that α_2-globulins in the plasma transport toxic substances to the kidneys where they are released and excreted. On an experimental basis it has also been suggested that the α_2-globulins have a detoxicating function in addition to their transport function (13). Since at least part of the toxic substances in urine might be glycoproteins and (or) glycopeptides the results of a recent investigation by Björnesjö *et al.* (15) are of interest in this connection. These authors found that the urinary excretion of both dialyzable and non-dialyzable hexosamine increased considerably after surgical operations and had already attained a maximum the day after the operation. The hexosamine in plasma increased more slowly and attained a maximum 3–7 days after the operation.

It has been known for a long time that urine contains protective colloids which might participate in the pathogenesis of renal calculi. The efficiency of the protective colloids can be measured by, for instance, the Donaggio reaction (cf. ref. 16). It is often much increased in pathological conditions such as infections, malignant neoplastic diseases, myocardial infarctions and collagen diseases. The protective colloids appear to have high carbohydrate content and are probably glycoproteins and (or) mucopolysaccharides (16–18).

Increased excretion of mucopolysaccharides in urine has been demonstrated in patients with gargoylism (19, 20), rheumatoid arthritis (21), lupus erythematosus (22), malignant neoplastic diseases (23), and during rapid growth in childhood (24). All of these states are characterized by abnormal or rapid metabolism of the connective tissues. It is accordingly most probable that this increased excretion reflects the metabolic condition of mucopolysaccharide-containing tissues. Except in the case of gargoylism, determination of the types of mucopolysaccharides excreted in these conditions does not seem to have been performed.

Brief survey of earlier investigations

In 1895 Mörner (25) showed that normal urine contained soluble protein in addition to an insoluble mucus substance (the nubecula). Part of this protein could be precipitated from dialyzed urine by acidifying with acetic acid and shaking with chloroform. The protein was precipitated as a complex with a substance which Mörner considered to be chondroitin sulphate.

Some 40 years later materials with blood-group activity were isolated from normal urine (26, 27).

The first electrophoretic study on non-ultrafilterable urine substances was published in 1951 by Rigas and Heller (28). They found fractions corresponding to the main protein fractions of serum. The globulin fractions were, however, not sharply defined and exceeded the albumin fraction in quantity. Other investigators have reported similar results (29–31). A rather poor separation has also been found in paper electrophoretic studies on high molecular weight urine materials (32, 16, 31). In addition, these studies have indicated a high content of carbohydrate in the urine materials, as evidenced by staining with the periodic acid-Schiff reagent. Recently, the plasma proteins in normal human urine have been examined qualitatively by means of immunoelectrophoresis (33–37). The excretion of 8 to 10 different components which react with antisera against normal human serum was demonstrated in these studies.

A few years ago Hamerman *et al.* (38) and King *et al.* (39) demonstrated that high molecular weight (non-dialyzable) materials in normal urine have a high content of different carbohydrates. Only one urinary glycoprotein appears to have been purified and (at least partly) characterized. This glycoprotein seems to occur in a series of polymers (40–42), one of which is the glycoprotein described by Tamm and Horsfall (43). The largest polymer has a molecular weight of 28×10^6 (44) and is four times as large at the Tamm-Horsfall glycoprotein.

Boyce and King *et al.* have tried different methods for fractionating non-ultrafilterable and ultrafilterable but non-dialyzable materials from normal urine, using, for example, Cohn's "Method 10" (45), acetone and ammonium sulphate (46) and also chromatography on DEAE[1]-cellulose (47). So far, however, they do not appear to have obtained homogeneous fractions with a characteristic composition. Anderson *et al.* (48) have fractionated non-ultrafilterable materials from normal urine on DEAE-cellulose. They also failed to obtain a separation in terms of characteristic and well-defined fractions. The fact that the largest portion of their material was treated with 0.1 *N* sodium hydroxide before chromatography might contribute in some measure to the poor separation attained by these workers. Starting with pooled urines from normal children, Bourrillon and Kaplan (49) have separated non-dialyzable materials by fractionation using different concentrations of ethanol. Urinary fractions precipitated in low ethanol concentrations were found to contain less carbohydrate than fractions precipitated in high ethanol concentrations. In a preliminary communication Gabriel *et al.* (50) have reported the fractionation of non-dialyzable materials from normal urine using iso-propanol, ether and continuous flow electrophoresis.

Confirmation of Mörner's observation that mucopolysaccharide mate-

[1] DEAE = di-ethyl-amino-ethyl.

rials occur in normal urine was given many years later by various authors (51–53) and in 1956 Di Ferrante and Rich (54) reported the isolation of chondroitin sulphate from normal urine. They stated that the chondroitin sulphate was not type B, but they were not able to provide further identification. In a later communication (21) Di Ferrante also presented evidence for the presence of hyaluronic acid in normal urine.

It may be concluded that, at present, knowledge concerning the proteins, glycoproteins and mucopolysaccharides in urine is very restricted. Further investigations of these substances in normal urine can be considered as a prerequisite for closer studies of their excretion in different physiological and pathological conditions. This consideration provided the starting-point for the present investigation.

The author's investigations

Fractionation of non-dialyzable proteins, glycoproteins and mucopolysaccharides in normal urine by ultrafiltration and by zone electrophoresis (I, II)

An initial problem in this investigations was the finding of a method suitable for concentrating proteins and carbohydrate-containing substances from large volumes of urine. Different ultrafiltration methods were tested. The low pressure method recently described by Everall and Wright (55) was found to give the best results and was finally adopted. This technique is fairly simple and rapid and gave good recoveries when used to concentrate the proteins from diluted sera (III). Visking dialysis tubes were used as ultrafiltration membranes. It has been shown by Craig (56) that Visking tubes of different sizes often possess very different permeabilities when used as dialysis membranes. The present author found this to be true for Visking tubes used as ultrafiltration membranes. Thus the amount of substance retained from the urine by the ultrafilter depended largely on the type of Visking membrane employed.

A method of separating non-dialyzable urinary substances into three fractions (*A, B* and *C*) with different mean molecular sizes was evolved on the basis of the different permeabilities of two types of Visking tubing. The fractionation was performed by means of two successive ultrafiltrations and a dialysis, as follows. A three or four litre aliquot of pooled normal male urine was passed through filter paper to remove insoluble material. The *A fraction* was separated from the filtered urine by an ultrafiltration membrane with high permeability, namely Visking

$^{8}/_{32}$ in. tubing. The *B fraction* was separated from the resulting ultrafiltrate by a membrane with low permeability, namely Visking $^{23}/_{32}$ in. tubing. An aliquot of this last ultrafiltrate, corresponding to about one litre of urine, was dialyzed through the $^{23}/_{32}$ in. tubing. The material which did not dialyze through this membrane constituted the *C fraction*. The permeabilities of the membranes used were determined with the aid of proteins (and peptides) of known molecular sizes. The A fraction appeared to contain all the large urine molecules down to a molecular weight range of 40,000–30,000 and included the usual plasma proteins. The membrane used for obtaining the B fraction seemed to retain all the molecules down to the range of 15,000–10,000. The C fraction appeared to contain molecules from the 15,000–10,000 range to less than 4,000.

The A, B and C fractions were analyzed for protein and different carbohydrates. The analyses were carried out according to known methods. An investigation was made into the application of the quantitative carbohydrate-analysis techniques to the analysis of the urinary fractions. Corrections were made for non-specific influences on the analyses, and the accuracy of the methods was determined. Paper chromatography after hydrolysis, and spectrophotometry revealed the presence of the following carbohydrates in the A, B and C fractions: glucosamine, galactosamine, galactose, mannose, fucose, *N*-acetylneuraminic acid, and uronic acid. In addition, the C fraction contained traces of glucose. The quantitative analyses showed that there were about the same distributions of protein and carbohydrate between the A, B and C fractions of different urines. The total amount of protein and carbohydrate, non-dialyzable through the Visking $^{23}/_{32}$ in. tubing, ranged from 213 to 244 mg per litre of pooled urine. The largest quantities of carbohydrate were present in the C fraction and the smallest quantities in the A fraction. This shows that in normal urine the greater parts of the glycoproteins and mucopolysaccharides consist of molecules with fairly small sizes. The B and C fractions were very carbohydrate-rich and appeared to contain more carbohydrate than protein, whereas the A fraction contained 2–3 times more protein than carbohydrate, clearly a consequence of its plasma protein content.

The A, B and C fractions were further separated by preparative zone electrophoresis at pH 8.6 on polyvinyl (Pevikon C-870) blocks. The distributions of protein and carbohydrate following electrophoresis were determined. The protein curves of the A fractions resembled that of serum, whereas the protein curves of the B and C fractions did not show distinct peaks. The relative amounts of the different carbohydrates assayed showed a considerable variation in different parts of the electrophoretic patterns. The A and B fractions contained materials with high sialic acid content having mobilities similar to that of albumin. Slowly moving or stationary fucose-rich materials corresponding to

small molecular sizes were present in the B and C fractions. The A and B fractions appeared to include at least three uronic acid-containing compounds which migrated faster than albumin. A fourth uronic acid-containing compound with a mobility similar to that of albumin was present in the A fractions. The electrophoretic patterns of the C fractions showed the presence of at least two broadly distributed uronic acid-containing compounds.

The soluble materials from pooled male and female urines, non-ultrafilterable through the membrane with the low permeability, namely the Visking $^{32}/_{32}$ in. tubing, were investigated further (II). These materials correspond to the sum of the A and B fractions described above. They were separated by preparative zone electrophoresis at pH's 8.6 and 4.5 and the distributions of protein and carbohydrates were determined. At both pH's the protein curves showed rather poor resolution, indicating a high degree of heterogeneity; however, they usually showed distinct albumin maxima. All protein fractions, including the albumin fraction, contained a considerable amount of carbohydrate.

In the experiments performed at pH 4.5 a large part of the urinary glycoproteins moved towards the anode faster than α_1-seromucoid which was determined on an adjacent part of the same Pevikon block. Accordingly it is probable that the urinary fraction with the highest mobility has an isoelectric point lower than 2.7, the value for α_1-seromucoid. This fast-moving fraction corresponded to one which in zone electrophoretic patterns obtained at pH 8.6 had the same or a slightly higher mobility than that of albumin. It had a high content of carbohydrate, especially of sialic acid, and a relatively small peptide content. In fact, this fraction contains about 30 per cent sialic acid which is more than in any known glycoprotein obtained from serum or urine (57).

At both pH's slowly moving or stationary fractions were particularly rich in fucose. The experiments performed at pH 8.6 indicated the presence of at least three mucopolysaccharide fractions moving faster than albumin. The fastest migrating fraction, which seemed to be obtained free of (glyco-)proteins, had approximately the same mobility as chondroitin sulphate, determined on an adjacent part of the same Pevikon block. Chemical analyses and infrared spectra revealed that this fraction was most probably a mixture of chondroitin sulphates A and C. The mobility of the slowest migrating fraction was similar to that of hyaluronic acid. A fourth mucopolysaccharide fraction migrated in the albumin region. It could be separated from albumin and sialic acid-rich glycoprotein(s) by repeated electrophoresis of the albumin fraction at pH 4.5.

No obvious differences were observed between electrophoretic patterns given by male and female urines. This fact seems to indicate that only small amounts of material originate from the genital tract.

Studies of the plasma proteins in normal human urine (III–V)

In the investigations just described (I, II) it was shown that all urine protein fractions obtained by electrophoretic separation contained considerable amounts of carbohydrate, generally more than in the corresponding plasma fractions. Accordingly, satisfactory information regarding the types and amounts of plasma proteins in normal urine cannot be obtained by electrophoresis alone. More precise qualitative and quantitative data regarding these proteins in normal urine can be obtained by the use of immunochemical methods. Therefore, a qualitative immunochemical study was undertaken in order to identify as many as possible of the plasma proteins excreted in urine from healthy individuals and to find out whether differences occurred between the urine proteins and corresponding plasma proteins. A quantitative study of the excretion of albumin in normal urine was also performed.

Qualitative examination of the plasma proteins in normal human urine (III)

The A and B fractions from normal urine (I) were examined for plasma proteins by means of double diffusion-in-gel and immunoelectrophoretic techniques. The following antisera were used in these experiments: rabbit and mule anti-total-human sera, rabbit and mule anti-total-human sera absorbed with purified plasma proteins, rabbit antisera against a number of purified plasma proteins, and rabbit anti-urine-protein sera.

The A fractions from pooled normal urines and from the urines of six male and six female healthy individuals were investigated. They were found to contain 14 to 20 different components which reacted with antisera against normal human plasma proteins. Twelve of these components were identified as the following plasma proteins: prealbumin, albumin, α_1-lipoprotein, α_1-seromucoid, ceruloplasmin, haptoglobin, α_2-macroglobulin, transferrin, β_{1A}-globulin, β_{2A}-globulin, fibrinogen and γ-globulin. In addition, three α_1-components, two α_2-components, one β_1-component, one β_2-component and one γ-component were found. One of the α_1-components was probably the main α_1-globulin (3.5 S). One of the two α_2-components appeared to be electrophoretically heterogeneous. No counterpart to this protein could be detected in plasma. The β_1-component was probably β_{1B}-globulin. The second component in the γ-region cross-reacted with plasma γ-globulin (7 S) and was antigenically deficient as compared to this protein. All 13 urine components tested gave reactions of immunological identity with corresponding plasma proteins. α_1-lipoprotein, ceruloplasmin, α_2-macroglobulin, β_{1A}-globulin and fibrinogen do not appear to have been identified previously in normal urine. The presence of haptoglobin in some normal urines seems to have been established in this investigation

for the first time by direct precipitation. Only one β_1-globulin, namely transferrin, appears to have been identified earlier in normal urine. In the present study, three β_1-globulins were found in all urines investigated. The diffusion-in-gel experiments with fibrinogen seemed to indicate that this protein was present in urine in a degraded state (in the form of products of fibrinogenolysis). The α_2- and β-lipoproteins and β_2-macroglobulin appeared to be absent from normal urine. This is in agreement with the findings of earlier investigators. However, traces of α_2-macroglobulin were found in some of the male and female urines. In view of the high molecular weight of α_2-macroglobulin (850,000) the presence of this protein in normal urine was rather unexpected although it is uncertain whether it was present in the native state.

The B fractions from some pooled normal urines were also examined for the presence of plasma proteins. Only one plasma-protein related component was found in the B fraction, namely a γ-globulin which gave a reaction of partial immunological identity with 7 S γ-globulin and was antigenically deficient as compared to this protein.

A γ-globulin of low molecular weight in normal human plasma and urine (IV)

It had been shown previously by Webb *et al.* (58) and by Franklin (59) that normal human urine contained low molecular weight γ-globulin fractions which cross-reacted with the γ-globulin fraction of plasma. As mentioned above, the present author found an ultrafilterable (and therefore apparently low molecular weight) γ-globulin in the B fraction of normal urines, that is, in concentrated urine ultrafiltrates. The B fraction appeared to be free from other plasma proteins. An attempt was made to find a similar γ-globulin in a corresponding fraction of normal plasma, as follows.—A 200 ml portion of pooled plasma and four 160–190 ml portions of individual fresh sera were diluted four times with physiological saline solution and then concentrated about 12 times by ultrafiltration through Visking $^{8}/_{32}$ in. tubes. The ultrafiltrates were concentrated to volumes of about 0.2 ml by further ultrafiltrations through Visking $^{23}/_{32}$ in. tubes. The concentrated plasma and serum ultrafiltrates (which corresponded to the urinary B fraction) were found to contain a protein, designated γ_L-globulin, which had the electrophoretic mobility of 7 S γ-globulin and which cross-reacted with this protein. The γ_L-globulin was antigenically deficient as compared to 7 S γ-globulin. Double diffusion-in-gel experiments showed that it had a higher diffusion coefficient than 7 S γ-globulin. For this reason and also because γ_L-globulin was ultrafilterable it can be assumed to have a lower molecular weight than the 7 S protein. The urine γ-globulin present in the B fraction gave a reaction of immunological identity with the plasma γ_L-globulin and behaved like this protein in double diffusion-

in-gel and immunoelectrophoretic experiments. Thus the γ-globulin component of urine probably originates from the plasma γ_L-globulin. A possible relationship between the γ_L-globulin and the Bence-Jones proteins was discussed.

Quantitative immunochemical determination of albumin in normal human urine (V)

The figures at present available for the quantities of albumin present in the high molecular weight materials of normal urines have been given on the basis of separation by moving boundary electrophoresis at pH 8.6. The urine materials were concentrated in different ways, namely ultrafiltration (28, 29), precipitation with alcohol (30) and dialysis combined with pervaporation (31). In these investigations, the figuers obtained for the average albumin content in 24-hour urine specimens from healthy individuals ranged between 14.8 and 31.0 mg. The present author found that the albumin region in electrophoretic separations at pH 8.6 contained glycoproteins and mucopolysaccharides in addition to albumin (1). Furthermore, it was demonstrated that the amount of these substances in the albumin region was dependent to a great extent on the method used for concentrating the urinary materials (1). Therefore, it could be presumed that all the albumin figures mentioned above were too high and that the considerable variation in these figures was due mainly to the fact that varying amounts of glycoproteins and mucopolysaccharides had contributed to the albumin boundary. For this reason, an investigation of the content of albumin in normal urine was made using a more specific, immunochemical method, namely the quantitative precipitin technique devised by Heidelberger *et al.* (cf. 60) with a turbidimetric modification (61).

The quantities of albumin in 24-hour urine specimens from healthy individuals, six male and six female, were determined by means of this method after concentration of the urines by ultrafiltration. The quantities obtained ranged between 3.6 and 14.1 mg with a mean value of 7.8 mg. The amounts of albumin in five 24-hour urine specimens from the same individual ranged between 8.6 and 14.7 mg with a mean value of 10.6 mg. No obvious correlation was observed between the albumin quantities and the volumes of the urine specimens.

The method used was tested in different ways. Thus purified urine albumin was prepared from four concentrated normal urines by successive zone electrophoretic separations at pH's 8.6 and 4.5. The precipitin curves of the urine albumin preparations were compared with the curve for purified plasma albumin. It was found that the curves for the various urine albumin preparations closely followed that for plasma albumin in the antibody excess zone. It is probable, therefore, that the quantitative determinations which were performed using this

part of the curve have given correct values. However, the curves for the urine albumin preparations were lower than that for plasma albumin in the equivalence zone, and even lower in the antigen excess zone. A possible reason for this phenomenon was discussed. A series of recovery experiments was performed. 94 to 99 per cent of the albumin added to urine or to ultrafiltered urine was recovered. The accuracy of the method appeared to be high, as shown in an experiment where the albumin quantities in five 1.0 litre portions of the same urine were determined.

General summary

The soluble high-molecular-weight substances in normal human urine were separated by two successive ultrafiltrations and one dialysis into three fractions with different mean molecular sizes. The three fractions were analysed for protein and different carbohydrates, were studied by double diffusion-in-gel and immunoelectrophoretic techniques and were further separated by preparative zone electrophoresis. The distributions of protein and carbohydrate following electrophoresis were determined. Three types of substances appeared to be present, namely proteins, glycoproteins and mucopolysaccharides.

It was found that the greatest part of the glycoproteins and mucopolysaccharides in normal urine consists of molecules with fairly small sizes. Urinary materials in the approximate molecular weight range from 40,000–30,000 to about 4,000 seemed to contain more carbohydrate than protein.

Most plasma proteins, except those with the largest molecular sizes, seemed to be excreted in normal urine. α_1-lipoprotein, ceruloplasmin, α_2-macroglobulin, β_{1A}-globulin and fibrinogen do not appear to have been identified previously. The presence of haptoglobin in some normal urines seems to have been established in this investigation for the first time by direct precipitation.

Normal human plasma was found to contain small amounts of an apparently low molecular weight γ-globulin (designated provisonally γ_L-globulin) which gives a reaction of immunological identity with a corresponding component in normal urine.

The quantities of albumin present in 24-hour urine specimens from healthy individuals were determined with the aid of a specific, immunochemical technique. The average albumin quantity found (7.8 mg) is much lower than the average quantities previously reported, all of which have been given on the basis of separation by moving boundary electrophoresis. These quantities are undoubtedly too high and vary considerably, generally as a result of the contribution to the albumin boundary of varying amounts of glycoproteins and mucopolysaccharides.

Two characteristic fractions were found among the materials of

glycoprotein type. One of these had a high content of carbohydrate, especially of sialic acid (about 30 per cent), and a relatively small peptide content. This fraction had a high mobility towards the anode at pH's 8.6 and 4.5. Another fraction which comprised substance(s) with fairly small molecular size(s), was characterized by a relatively high content of fucose. Electrophoretically it appeared to be slowly moving or stationary both at pH 8.6 and 4.5.

The presence in normal urine of at least four mucopolysaccharide fractions was demonstrated. One of these is probably a mixture of chondroitin sulphates A and C. A second fraction might be hyaluronic acid in view of its electrophoretic mobility.

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